A Study of U.S. Orchards To Identify Potential Sources of *Escherichia coli* O157:H7[†]

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ABSTRACT

The association of unpasteurized apple cider with *Escherichia coli* O157:H7 foodborne illness has led to increased interest in potential reservoirs of this pathogen in the orchard. Fourteen U.S. orchards were surveyed in autumn 1999 to determine the incidence and prevalence of *E. coli* O157:H7, *E. coli,* total aerobic microflora, and yeasts and molds. Fruit samples ($n = 63$) (eight apple and two pear varieties) and soil, water, and fecal samples were collected. Samples were plated on (i) tryptic soy agar for total mesophilic aerobic count, (ii) *E. coli* and coliform Petrifilm for total coliforms and *E. coli,* and (iii) yeast and mold Petrifilm. Samples positive for coliforms and *E. coli* were enriched and tested for *E. coli* O157:H7. Fruit was also tested for internalization of microflora by aseptically removing the core, stem, and calyx areas, and the individual sections were assessed for the categories of microflora listed above. *E. coli* was detected in soil and water and in 6% of fruit samples (three pear samples and one apple sample), generally collected from areas previously designated as high risk in this study. However, no *E. coli* O157:H7 was found. Coliforms were found in 74% of fruit samples and were internalized in the cores of 40% of fruit tested. Yeasts and molds were internalized in 96.7% of samples and aerobic bacteria in 89.6%. *E. coli* was not found to be internalized. Total aerobic counts and total coliforms were higher in dropped and damaged fruit ($P < 0.05$). Findings suggest that dropped or damaged fruit should not be included in fruit designated for the production of unpasteurized juice or for the fresh or fresh-cut market. In addition, orchards should be located away from potential sources of contamination, such as pastures.

Produce, including unpasteurized fruit juice, has been established as a vector for foodborne illness *(21)*. In 1980, unpasteurized apple cider was linked to foodborne illness in Canada *(27)*, most probably attributable to *Escherichia coli* O157:H7, though this organism was not definitively linked to foodborne disease until 1982 *(22)*. Since 1991, there have been several outbreaks of foodborne disease associated with *E. coli* O157:H7 in unpasteurized apple cider *(2–5)*. These outbreaks were particularly significant, as they occurred in a highly acidic food product, previously thought to be safe because of its low pH. However, studies have shown *E. coli* O157:H7 to survive in unpasteurized apple cider produced in the traditional manner *(19, 33)*. The fact that this is a ready-to-eat product, receiving no further processing before consumption, is a matter of concern. Consequently, the Food and Drug Administration has stated that fresh juice products must be treated with a process designed to yield a 5-$\log_{10}$ reduction in the most resistant organisms of public concern *(10,11)*. The presence of pathogens such as *E. coli* O157:H7 on the surface of fruit also has implications for the safety of supplies to the fresh and fresh-cut fruit markets.

In this study, potential reservoirs for *E. coli* O157:H7 in the orchard environment were investigated. The microflora profile on fruit harvested from a given orchard will be affected by orchard management practices. Constituents of the orchard environment—including fecal matter, soil, irrigation and surface water, and windblown dust—are potential contamination sources for fruit. However, mechanisms of contamination are speculative, and further investigation is required before appropriate interventions can be introduced to reduce the risk of contamination. A survey of fruit, orchard environments, and orchard management practices was performed to identify any associations between management practices and the prevalence and profile of microflora on fruit and in the orchard environment. While the presence of *E. coli* does not necessarily indicate that *E. coli* O157:H7 is present *(20)*, it was chosen as an appropriate indicator for this organism, as presumably it will be disseminated in the same manner as *E. coli* O157: H7.

MATERIALS AND METHODS

Collection of fruit. Samples were collected on 20 through 24 September 1999 at 14 orchards throughout the United States: eight in the Pacific Northwest, one in the Midwest, and five in the Northeast (Table 1). Apples were collected at 12 orchards, and pears were collected at two orchards. Apples and pears were frequently grown in the same locality; therefore, both types of orchards were subject to similar contamination hazards. Apples collected were grown for both cider and fresh markets, while pears collected were grown for the fresh market only. Twelve of the

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† Mention of brand or firm names does not constitute and endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE 1. *Microbial populations (log$_{10}$ CFU/g) of fruit collected from U.S. orchards, autumn 1999*

Location	Name	Variety (*n*)	Total aerobic count	Total coliforms	Yeasts and molds
Pacific NW	1[a,b]	Red Delicious (18)	3.94 (1.27) ABC[c]	0.71 (1.03) A	4.66 (0.56) ABC
Pacific NW	2[a]	Gala (20)	4.93 (1.04) A	0.86 (0.83) A	4.82 (0.56) A
Pacific NW	3	Golden Delicious (20)	3.97 (1.01) ABC	0.50 (0.74) A	4.02 (0.66) CDE
Pacific NW	4[b]	Red Delicious (20)	4.14 (0.92) ABC	1.58 (1.06) A	4.26 (0.50) ABCDE
Pacific NW	5[b,d]	Granny Smith (17)	5.03 (1.43) A	1.48 (1.30) A	4.59 (0.65) ABCD
Pacific NW	6	Fuji (20)	4.88 (1.38) A	1.02 (1.26) A	4.13 (0.46) BCDE
Pacific NW	7[b,e]	D'Anjou (pears) (12)	3.81 (2.72) ABC	1.53 (1.43) A	4.27 (0.77) ABCDE
Pacific NW	8[d,e]	Bosc (pears) (11)	2.93 (1.79) BC	0.63 (0.82) A	4.24 (0.44) ABCDE
Midwest	9[b,e]	Golden Delicious (14)[f] and Granny Smith (14)	3.85 (1.11) ABC	1.12 (1.00) A	ND[g]
Northeast	10[b,d]	McIntosh (20)	4.02 (1.03) ABC	1.67 (1.79) A	3.93 (0.66) DE
Northeast	11[b,d,e]	Golden Delicious (10)	2.73 (0.88) C	0.33 (0.62) A	3.92 (0.15) DE
Northeast	12[b,e]	Empire (8) and Red Delicious (8)	4.40 (0.67) AB	1.15 (1.22) A	4.02 (0.10) CDE
Northeast	13[b,d,e]	Cortland (6)	5.29 (0.36) A	1.77 (1.39) A	4.76 (0.90) AB
Northeast	14[b,d,e]	Cortland (6) and Empire (6)	3.68 (0.76) ABC	1.05 (1.10) A	3.91 (0.11) E

[a] Organically managed orchards.

[b] Irrigation water collected.

[c] Data obtained using whole blend preparation method. Means in each column followed by different letters are significantly different ($P < 0.05$). Standard deviations are given in parentheses.

[d] Fecal matter collected.

[e] Designated high-risk orchards.

[f] *E. coli* located on calyx down Golden Delicious apple.

[g] ND, not done.

orchards visited were conventionally managed, with the remaining two orchards (orchards 1 and 2; both apple orchards) organically managed. One of the organic orchards visited in this survey was fertilized with composted manure, primarily chicken manure and other organic wastes. The other orchard was fertilized by an alfalfa cover crop.

A total of eight different apple varieties (Cortland, Empire, Fuji, Golden Delicious, Granny Smith, Gala, McIntosh, and Red Delicious) and two pear varieties (Bosc and D'Anjou) were collected. In orchards 9, 12, and 14, two different cultivars were collected (Table 1). These were analyzed separately. The orchards visited were designated as "high risk" or "low risk," based on observations made at the time of sampling. Those that were designated high risk included orchards that had previously yielded samples positive for *E. coli* (orchards 9, 11, 12, 13, and 14), those that had excessive fecal matter in the orchard (orchards 7, 8, and 12), and those that were irrigated with nonpotable water (orchard 11). The other seven orchards visited did not have any of these risk factors and were therefore deemed low risk.

A total of five categories of fruit, including tree fruit and dropped fruit, were collected. Fruit picked from the tree was designated either "calyx (i.e., blossom end) down" or "calyx up." By collecting fruit that had grown calyx up, it could be determined if fruit oriented in this way had significantly greater microflora populations due to increased exposure of the calyx channel to potential sources of contamination from dust, contaminated water, etc., than fruit that had grown calyx down. Pears do not commonly grow with the calyx oriented upwards, so this category was not included for this fruit. Fruit designated "damaged on tree," which had evidence of damage from bird pecks, hail, or splitting during growth, etc., was collected. Dropped fruit, including intact drops (designated "drops") and partially decayed drops (designated "drops with decay"), was collected. Not all five categories of fruit were collected at each orchard, due to limited availability in some

locations. A total of 63 fruit samples, comprising samples from all five categories described, were collected. Each sample consisted of 24 pieces of fruit, divided into four composites of six, packed in individual polyethylene Ziploc bags. Samples were packed in fruit boxes and transported to the Eastern Regional Research Center within 3 days. Fruit was stored at 4°C until analysis.

Determination of microflora populations. All 63 fruit samples were tested for microflora populations on the whole fruit. The microflora population was estimated by the "whole blend" method, using two duplicate composite bags of six apples or pears. Six pieces of fruit were weighed, and each was cut in four on a sterile cutting board. The fruit was placed in a stainless steel blender (4-liter Waring blender; Waring, New Hartford, Conn.), combined with an equal volume (wt/vol) of sterile 0.1% peptone water (PW; Difco Laboratories, Detroit, Mich.), and blended on medium speed for 1 min. This procedure was repeated for the second composite bag of fruit. Of the 63 fruit samples collected, 45 were tested for internalization of microflora. Two composite samples of six apples or pears were used for this procedure. The skin areas around the stem and calyx of each piece of fruit were removed using a sharp, sterile flamed knife to prevent cross-contamination from the outer skin area to the interior of the apple. A sterile cork borer (27 mm in diameter) was then pushed into the fruit from the stem end, and the core was removed. The remainder of the fruit was discarded. The cored portion of the fruit was placed on a sterile cutting board and divided into stem, core, and calyx portions using a sterile knife. Samples consisting of six stem, core, and calyx portions were diluted in four parts PW (wt/vol) and individually blended in a glass blender (2-liter Waring blender) on medium speed for 1 min. This procedure was performed in duplicate for each set of six stem, core, and calyx portions.

Blended samples were filtered through a filter bag (Spiral Biotech, Bethesda, Md.). Approximately 60 ml fruit filtrate (two

30-ml duplicate samples) from each blended sample was collected. Fruit filtrate samples (1.1- or 0.1-ml aliquots) were diluted as necessary in 9.9-ml volumes of PW, with the remainder of the filtrate retained at 4°C.

Samples were enumerated for total mesophilic aerobic counts, total coliforms, *E. coli*, and yeasts and molds. Total mesophilic aerobic counts were estimated by plating 0.1-ml aliquots on Trypticase soy agar (TSA; Difco) using a spiral plater (Autoplate 4000; Spiral Biotech). One-milliliter aliquots were manually plated when increased sensitivity was required. TSA plates were incubated at 35°C for 24 h and manually counted. An automated counting system was not appropriate due to the many different morphologies of the colonies that were observed in this study.

E. coli and total coliform counts were estimated by plating 1-ml aliquots on *E. coli* and coliform count Petrifilm plates (3M, St. Paul, Minn.). Plates were incubated at 35°C and examined at 24 and 48 h for the presence of coliforms (red colonies with gas) and *E. coli* (blue colonies with gas). Samples displaying these types of colonies were enriched to determine if they were positive for *E. coli* O157:H7. Briefly, the enrichment procedure was as follows [11]: fruit filtrate samples (50 ml) were incubated in Trypticase soy broth (200 ml; Difco) supplemented with Tween 20 (0.6% vol/vol) (Sigma, St. Louis, Mo.). Samples were incubated and shaken (100 rpm) at 37°C for 4 h. At this point, novobiocin (Sigma) was added to each sample at a final concentration of 0.02%, and the samples were incubated for a further 20 h. The enriched samples were streaked (1 μl) onto sorbitol MacConkey agar (Becton Dickinson, Cockeysville, Md.) (supplemented with cefixime-tellurite supplement: cefixime [0.05 mg/liter] and potassium tellurite [2.5 mg/liter]; Dynal Inc., Lake Success, N.Y.) and incubated for 24 h at 35°C. Suspected O157:H7 colonies, which appeared colorless on sorbitol MacConkey supplemented with cefixime-tellurite, were transferred to slants of TSA supplemented with 0.6% yeast extract (TSAYE; Difco) and incubated overnight at 35°C. After incubation at 35°C for 24 h, growth on TSAYE was tested for the production of indole by the spot test using filter paper wetted with Kovak's reagent (Difco). Indole-positive isolates were tested for the O157 antigen with the RIM *E. coli* O157:H7 latex test (Remel, Lenexa, Kans.).

Yeast and mold populations were estimated by plating 1-ml aliquots on yeast and mold count Petrifilm plates (3M). Plates were kept at room temperature (approximately 25°C) and counted manually at days 3 and 5.

Environmental samples collected. Four soil samples (approximately 100 g) were collected from the perimeter and the interior of orchards 1 to 10. Duplicate soil samples (10 g) were diluted in PW (90 ml), mixed using a stomacher (Seward 400 circulator; Seward Ltd., London, UK) for 1 min on medium speed, and filtered through a filter stomacher bag (Spiral Biotech). The resultant filtrate was diluted in PW as necessary and plated on TSA and *E. coli* and coliform count Petrifilm for the enumeration of total mesophilic aerobic counts, total coliforms, and *E. coli.*

Four irrigation water samples (approximately 10 ml) were collected from each of 9 of the 14 orchards visited (Table 1). Water samples were diluted in PW as necessary and plated on TSA and *E. coli* and coliform Petrifilm for the enumeration of total mesophilic aerobic counts, total coliforms, and *E. coli.*

Fecal matter (approximately 100 g) was collected from six of the orchards visited (Table 1). Duplicate samples (25 g) were added to Trypticase soy broth (225 ml) supplemented with Tween 20 (0.6% vol/vol) and the enrichment procedure for *E. coli* O157:H7, performed as described above.

Statistical analysis. Analysis of variance, performed using SAS (24), was used to determine the effect of the orchard, fruit category, and fruit portion on microflora numbers. The Bonferroni Least Square Difference method was used to determine differences in microflora populations between different categories of fruit in high- and low-risk locations. Means and standard deviations were determined using commercial spreadsheet software (Excel 97; Microsoft, Redmond, Wash.).

RESULTS

Presence of *E. coli* in fruit. Table 1 shows the microbial populations of fruit collected from U.S. orchards, autumn 1999. These data reflect results obtained using the whole blend preparation method. No *E. coli* O157:H7 was detected in any fruit or environmental sample tested. *E. coli* was detected in 6.3% (*n* = 4) of fruit samples tested. Three of the four samples that were positive for *E. coli* were pears that had been damaged in some way. Two separate fruit samples (both pears) from orchard 8, one damaged on tree and one drop with decay sample, were positive for *E. coli* (populations, 0.40 and 0.70 log$_{10}$ CFU/g, respectively). One drop with decay sample (pear) from orchard 7 was positive for *E. coli* (population, 0.85 log$_{10}$ CFU/g). An intact tree-picked apple from orchard 9 was also positive for *E. coli* (1.19 log$_{10}$ CFU/g).

Effect of orchard on microflora population. Orchard 13 had the highest overall total mesophilic aerobic count (5.29 log$_{10}$ CFU/g) for whole fruit. There was much evidence of deer in this location, which was therefore designated high risk. Interestingly, total aerobic counts in orchard 13 were significantly greater than only one other orchard (orchard 11) (*P* < 0.05) (Table 1), which was also designated high risk. This again demonstrates that orchards in which risk factors were identified did not consistently have the highest microflora populations.

Coliforms were detected in 74.6% of fruit samples tested. There was no significant difference in total coliform numbers between the orchards visited (range, 0.33 to 1.77 log$_{10}$ CFU/g) (Table 1).

Yeast and mold counts were significantly higher (*P* < 0.05) (4.82 log$_{10}$ CFU/g) in one of the organic orchards (orchard 2) than in orchards 3, 6, 9, 11, 12, and 14 (Table 1), probably due to the proliferation of these microflora in the absence of fungicides.

There was no significant difference in mean total aerobic counts (4.46 versus 4.04 log$_{10}$ CFU/g) or mean total coliform numbers (0.80 versus 1.14 log$_{10}$ CFU/g) between organic and conventionally managed orchards, respectively.

Although the orchards where *E. coli* was detected in fruit were high-risk locations, overall microbial populations were not significantly higher in these orchards than in orchards designated low risk (Tables 2 and 3). The designation of high- and low-risk status on the orchards visited did not consistently mirror the pattern of microbial contamination on the fruit, with those orchards designated as high risk often having significantly lower overall microflora populations than the other orchards (Table 2). Exceptions included coliforms in tree-damaged fruit, which were significantly higher in high-risk orchards (2.68 versus 0.63 log$_{10}$

TABLE 2. *Microbial populations (log$_{10}$ CFU/g) based on high- or low-risk designation*

Data set	Microflora category	High risk	Low risk
Whole blend data	Total aerobic count	3.59 (1.55) A ($n = 85$)[a]	4.41 (1.17) B ($n = 151$)
Whole blend data	Total coliforms	1.03 (1.12) A ($n = 88$)	1.13 (1.23) A ($n = 148$)
Whole blend data	Yeasts and molds	4.17 (0.57) A ($n = 66$)	4.31 (0.63) B ($n = 151$)
Soil data	Total aerobic count	5.71 (1.17) A ($n = 25$)	5.75 (0.24) A ($n = 52$)
Soil data	Total coliforms	2.51 (1.15) A ($n = 28$)	1.23 (1.02) B ($n = 52$)
Soil data	*E. coli*	0.15 (0.41) A ($n = 28$)	0.11 (0.50) A ($n = 56$)
Internalization data	Total aerobic count	3.13 (1.77) A ($n = 107$)	3.68 (1.65) B ($n = 399$)
Internalization data	Total coliforms	0.88 (1.47) A ($n = 114$)	0.91 (1.13) A ($n = 412$)
Internalization data	Yeasts and molds	3.81 (1.51) A ($n = 112$)	4.30 (1.31) B ($n = 394$)

[a] Means in each row followed by different letters are significantly different ($P < 0.05$). Standard deviations are given in parentheses. The number of values that comprise each mean is included.

CFU/g)—though this might be a consequence of the disparity in sample sizes. Also, yeasts and molds were significantly higher in dropped fruit in high-risk (4.84 log$_{10}$ CFU/g) than in low-risk (3.96) orchards (Table 3).

Effect of fruit category collected. Table 4 lists the microbial populations of fruit, based on the five categories collected, i.e., calyx up, calyx down, damaged on tree, dropped, and dropped with decay. Of these five categories of fruit, drops with decay had significantly higher total counts (5.94 log$_{10}$ CFU/g) ($P < 0.05$), total coliforms (2.08 log$_{10}$ CFU/g) ($P < 0.05$), and yeasts and molds (5.11 log$_{10}$ CFU/g) ($P < 0.05$) than any other category collected. Intact dropped fruit had significantly higher total counts (4.47 log$_{10}$ CFU/g) ($P < 0.05$) and total coliforms (1.74 log$_{10}$ CFU/g) ($P < 0.05$) than tree fruit. Damaged tree fruit had significantly higher total counts (4.42 log$_{10}$ CFU/g) ($P < 0.05$) than intact tree fruit (i.e., calyx up and calyx down samples). Tree-picked fruit had significantly lower total aerobic counts, total coliforms, and yeasts and molds ($P <$

0.05) and less microflora present in the core of the fruit ($P < 0.05$) than dropped or damaged fruit. Only 59.3% of intact tree fruit were positive for total coliforms, compared to 83.9% of damaged on tree, dropped, and dropped with decay samples (data not shown). There was no significant difference between microflora counts on intact tree fruit oriented calyx up or calyx down.

Evidence of internalization of microflora. Table 5 shows the microbial populations of the stem, core, and calyx sections of this fruit. There were significantly higher total mesophilic aerobic counts ($P < 0.05$), total coliform counts ($P < 0.05$), and yeast and mold counts ($P < 0.05$) in the stem and calyx sections than in the core. Overall, greater numbers of total mesophilic aerobic flora and total coliforms were internalized within dropped and damaged fruit (4.33 and 1.47 log$_{10}$ CFU/g, respectively) than within intact tree fruit (1.36 and 0.10 log$_{10}$ CFU/g, respectively) ($P < 0.05$). There was no significant difference between the number of microorganisms internalized in tree fruit ori-

TABLE 3. *Microbial populations (log$_{10}$ CFU/g) in different categories of fruit based on high- or low-risk designation*

Category	Total aerobic count	Total coliforms	Yeasts and molds
Calyx down			
High risk	2.83 (1.26) A[a] ($n = 24$)	0.46 (0.83) A ($n = 24$)	3.88 (0.19) A ($n = 20$)
Low risk	3.31 (0.92) A ($n = 37$)	0.45 (0.66) A ($n = 38$)	4.09 (0.39) A ($n = 40$)
Calyx up			
High risk	3.45 (1.01) A ($n = 24$)	1.00 (1.04) A ($n = 24$)	3.89 (0.11) A ($n = 12$)
Low risk	3.87 (1.01) A ($n = 28$)	0.39 (0.70) A ($n = 28$)	4.20 (0.61) A ($n = 28$)
Damaged on tree			
High risk	3.98 (1.03) A ($n = 4$)	2.68 (1.04) A ($n = 4$)	3.68 (0.13) A ($n = 4$)
Low risk	4.48 (1.14) A ($n = 31$)	0.63 (0.97) B ($n = 28$)	4.36 (0.53) A ($n = 32$)
Drops			
High risk	4.83 (1.59) A ($n = 22$)	1.34 (1.12) A ($n = 22$)	4.84 (0.74) A ($n = 12$)
Low risk	4.26 (0.88) A ($n = 38$)	1.96 (0.93) A ($n = 40$)	3.96 (0.43) B ($n = 40$)
Drops with decay			
High risk	—[b]	2.23 (1.25) A ($n = 4$)	5.30 (0.05) A ($n = 2$)
Low risk	5.94 (0.83) ($n = 28$)	2.05 (1.55) A ($n = 24$)	5.09 (0.46) A ($n = 30$)

[a] Means in each column for each category followed by different letters are significantly different ($P < 0.05$). Standard deviations are given in parentheses.
[b] —, no data collected.

TABLE 4. *Effect of fruit category collected on microbial populations (log_{10} CFU/g)*

Category (n)	Total aerobic count	Total coliforms	Yeasts and molds
Calyx down (61)	3.12 (1.08) A[a]	0.46 (0.72) A	4.02 (0.35) A
Calyx up (52)	3.67 (1.02) A	0.67 (0.92) A	4.10 (0.53) A
Damaged on tree (60)	4.42 (1.13) B	0.88 (1.17) A	4.29 (0.55) A
Drops (28)	4.47 (1.21) B	1.74 (1.04) B	4.17 (0.63) A
Drops with decay (35)	5.94 (0.83) C	2.08 (1.49) B	5.11 (0.44) B

[a] Data obtained using whole blend preparation method. Means in each column followed by different letters are significantly different ($P < 0.05$). Standard deviations are given in parentheses.

ented calyx up or calyx down. Coliforms were detected in 40% of core samples, in contrast to 64.4% of calyx samples and 73.3% of stem samples tested. *E. coli* was internalized within the core of one dropped decayed sample. This was probably an artifact of the actual piece of fruit. It was observed that dropped decayed fruit was often decomposed, making exact distinctions between stem, core, and calyx portions very difficult.

Microbial populations in the orchard environment. Table 6 shows the microflora populations of the soil and irrigation water samples collected. *E. coli* was detected in soil samples from orchards 3, 6, 7, and 9, i.e., in 4 of 10 orchards where soil samples were collected. Total mesophilic aerobic counts in soil samples collected from the orchards visited ranged from 4.86 (orchard 8) to 6.67 log_{10} CFU/g (orchard 7). The high count observed in orchard 7 might be a result of the fertilization of this orchard with cow manure. Orchard 9 had significantly higher total coliforms in the soil (4.31 log_{10} CFU/g) than the other orchards tested ($P < 0.05$). This high-risk orchard was within close proximity of a pasture. Both orchards 7 and 9 were associated with the presence of *E. coli* on fruit.

Irrigation water samples from three of the orchards where water was collected, i.e., orchards 9, 11, and 13, were positive for *E. coli*, with populations ranging from

<0.18 (the lower limit of detection) to 0.75 log_{10} CFU/ml (Table 6).

E. coli O157:H7 was not detected in any of the fecal samples collected.

DISCUSSION

E. coli O157:H7 was not detected in any of the locations visited, underlining the low incidence of this organism in the environment. It is possible that a greater number of positive samples would have been detected if the collection time had fallen between mid-October and mid-November, reported by Dingman (6) as a period when a higher incidence of *E. coli* contamination in apples is observed. However, data relating to the incidence and prevalence of *E. coli* in the orchard provide useful information for those compiling quantitative risk assessments for *E. coli* O157:H7 in unpasteurized apple cider. This study identifies critical control points within the orchard by recognizing areas where there is increased potential for contamination; therefore, the findings also have implications for the fresh apple and pear markets. Potential risk factors observed in locations visited included the presence of fecal matter, from animals or from direct application of manure, proximity to pasture lands, and irrigation with nonpotable water (9, 29). The soil and water from some orchards were contaminated with *E. coli*.

TABLE 5. *Microbial populations (log_{10} CFU/g) of the calyx, core, and stem sections of the five fruit categories sampled*

Category	Fruit section (n)	Total aerobic count	Total coliforms	Yeasts and molds
Calyx down	Calyx (38)	3.34 (1.30) A[a]	0.60 (1.06) A	5.04 (0.55) A
	Core (38)	1.37 (1.15) B	0.10 (0.45) A	2.14 (1.00) B
	Stem (38)	3.32 (1.46) A	0.32 (0.62) A	4.51 (0.61) A
Calyx up	Calyx (30)	3.94 (0.84) A	0.48 (0.92) A	5.06 (0.58) A
	Core (30)	1.36 (1.03) B	0.09 (0.38) A	2.53 (1.16) B
	Stem (32)	3.11 (1.01) A	0.87 (1.09) A	4.48 (0.69) A
Damaged on tree	Calyx (34)	4.12 (1.07) A	1.02 (1.18) A	4.97 (0.46) A
	Core (32)	2.32 (1.66) B	0.64 (1.11) A	2.38 (1.25) B
	Stem (34)	4.18 (1.54) A	1.44 (1.24) A	4.61 (0.54) A
Drops	Calyx (36)	4.58 (1.15) A	1.69 (1.23) A	5.20 (0.36) A
	Core (34)	2.39 (1.16) B	0.40 (0.84) B	2.61 (1.06) B
	Stem (36)	4.66 (0.94) A	1.53 (1.11) A	4.77 (0.57) A
Drops with decay	Calyx (33)	5.33 (0.80) A	1.60 (1.14) A	5.26 (0.70) A
	Core (30)	4.33 (1.14) A	1.47 (1.07) A	3.96 (0.91) B
	Stem (31)	5.23 (1.05) A	1.50 (0.99) A	5.36 (0.56) A

[a] Means in each column, within each category, followed by different letters are significantly different ($P < 0.05$). Standard deviations are given in parentheses.

TABLE 6. *Microbial populations (log$_{10}$ CFU/g) of soil and irrigation water collected*

Location	Name	Soil				Irrigation water			
		n	Total aerobic count	Total coliforms	E. coli	n	Total aerobic count	Total coliforms	E. coli
Pacific NW	1[a]	8	5.79 BC[b]	1.81 BCDE	nd[c] A	4	0.50	nd	nd
Pacific NW	2[a]	8	5.83 BC	1.85 BCDE	nd A		—[c]	—	—
Pacific NW	3	8	5.69 BC	1.01 DEFG	0.13 A		—	—	—
Pacific NW	4	8	6.07 B	nd G	nd A	4	1.75	nd	nd
Pacific NW	5	8	5.55 C	0.42 GF	nd A	4	0.16	nd	nd
Pacific NW	6	8	5.63 BC	2.27 BCD	0.65 A		—	—	—
Pacific NW	7[d]	7	6.67 A	2.79 B	0.21 A	4	1.75	0.48	nd
Pacific NW	8[d]	6	4.86 D	1.47 CDEF	nd A		—	—	—
Midwest	9[d]	8	5.68 BC	4.31 A	0.32 A	4	3.34	0.74	≤0.18[e]
Northeast	10	4	5.61 C	0.67 EFG	nd A		—	—	—
Northeast	11[d]		—	—	—	4	2.96	0.45	≤0.18
Northeast	12[f] to 14[d]		—	—	—	4	4.20	4.20	0.75

[a] Organically managed orchards.
[b] Means in each column followed by different letters are significantly different ($P < 0.05$).
[c] nd, not detected; —, analysis not performed.
[d] Designated high-risk orchards.
[e] 0.18 CFU was the lower limit of detection for this technique.
[f] Sample taken from creek that supplies irrigation water to all these orchards.

However, neither the presence of *E. coli*, nor the risk factors mentioned, was correlated with increased microbial populations on the fruit in the present study, i.e., orchards designated high risk did not have significantly higher numbers of microflora than low-risk orchards. This was probably an artifact of the size of the study and highlights the difficulties associated with interpreting the results from a survey such as this.

Coliforms were widespread in the locations visited. However, the enumeration medium used in this study, i.e., *E. coli* and coliform Petrifilm, does not distinguish between fecal and nonfecal coliforms, and only "total coliforms" could be enumerated. It is not uncommon to find coliforms on fresh and minimally processed vegetables (7, 26, 32). However, the presence of such coliforms in produce, even in high numbers, does not indicate that these products pose a health hazard (20). Therefore, any observed increase cannot be linked to an actual source of fecal coliforms, though it may be possible to associate an increase in total coliforms with an increased potential for contamination.

Intact tree fruit had significantly lower counts than the other fruit samples collected. The hypothesis that the orientation of the fruit on the tree might be associated with the potential for internalization of microflora was not borne out. However, tree fruit was associated with *E. coli* contamination on two occasions (one intact and one damaged sample). Previously, it had been thought that contamination of fruit with *E. coli* was a consequence of contact with the ground or humans, as this organism is rarely found on plants in nature (28). It is possible that *E. coli* contamination of tree fruit may come from birds; Wallace et al. (30) reported that birds, mainly gulls, can harbor *E. coli* O157: H7. Further studies are ongoing at this laboratory to char-

acterize situations in which *E. coli* can contaminate tree fruit.

This study confirms that dropped and damaged fruit have increased microbial populations and that they are a potential source of *E. coli*. A study conducted in the New England area in 1991 indicated that all apple cider producers surveyed used drop apples (2); however, it is important to remember that this survey took place before most of the larger outbreaks occurred. Wright et al. (31) indicated that 32% of apple cider producers surveyed in 1998 in Virginia used drop apples, while Uljas and Ingham (29) found that only 14% of producers surveyed used drops during the 1998 to 1999 cider production season. Luedtke and Powell (17) reported that none of the Ontario cider manufacturers surveyed used dropped apples for the production of unpasteurized apple cider. There is much evidence to show that *E. coli* O157:H7 can grow in areas of injury and decay (23) and areas inaccessible to washing (1, 24) on apples. Wright et al. (31) reported that 37.5% of apple cider producers in Virginia, surveyed in 1998, included damaged fruit in cider production. To improve the safety of the product, unpasteurized juice should be made from intact tree fruit only. It is also advisable to remove drops from the orchard floor periodically, to reduce grazing by large animals, and to control the insect population, which has been shown to be a potential vector of *E. coli* O157:H7 (13–16). It should be noted that Dingman (6) reported that *E. coli* contamination of cider could not be attributed to the use of drops or to the handling by workers but rather, was a consequence of "a common unidentified factor(s) that occurred in the production of cider." However, without knowing for certain what such factors might be, it would appear prudent to suggest that producers promote stringent efforts among pickers

to eliminate all fruit that has been in contact with the ground, or shows signs of decay, from that destined for nonthermally treated juice or the fresh or fresh-cut market *(29)*. It is important that all producers are aware of the significant risks associated with the inclusion of drops during processing, and there is thus a need for effective risk communication in this area.

The irrigation water in three of the high-risk orchards was contaminated with *E. coli*. This is an obvious route of contamination for all fruit. Potable water should be used for irrigation wherever possible, with nonpotable water closely monitored for the presence of *E. coli* and treated, if necessary. Wright et al. *(31)* indicated that 72.5% of unpasteurized apple cider producers in Virginia regularly tested their water supply for coliforms.

This study found no difference in microbial populations between conventionally and organically managed orchards. The organically farmed orchards were well managed, did not use cow manure as fertilizer, and were not designated as high risk. However, only two organic farms were surveyed, and sampling at more such orchards is necessary to identify any differences between fruit from organically and conventionally managed orchards.

Identification of potential vectors for microflora transmission provides helpful supporting data for use in hazard analysis critical control point procedures and risk assessment models for unpasteurized cider production, by identifying critical control points for contamination within the system. Using the information from this study, interventions can be developed to reduce the potential for contamination of fruit in the orchard, thereby improving the safety of unpasteurized apple cider and the fresh and fresh-cut fruit supply. Proximity to pasture was cited as a risk factor when designating orchards as high or low risk. Samples positive for *E. coli* were collected from orchards that were situated close to pastures. Also, this study has shown that soil can frequently be contaminated with *E. coli,* albeit at low levels. Other work performed in this group detected *E. coli* in 12% of a small sample (*n* = 42) of apples picked from trees in an orchard located near a cattle feed lot *(8)*. However, it may be difficult in practice to locate all orchards away from pastures—Uljas and Ingham *(29)* indicated that 46% of orchards in their study were within 0 to 0.5 miles of farms with domestic animals, primarily cows. Growers should endeavor to limit the potential for fecal contamination of their orchards, perhaps by erecting fences to keep animals out *(18)*. Wright et al. *(31)* indicated that 8% of processors in Virginia use manure to fertilize their orchards, and 5% permit domestic animals to graze in the orchards. All indications are that these are not advisable practices. Recommendations as to the appropriate "buffer" zone between an orchard and a pasture area cannot be made at this point, and studies are ongoing to characterize the risk associated with locating an orchard beside a pasture area.

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REFERENCES

1. Annous, B. A., G. M. Sapers, A. M. Mattrazzo, and D. C. R. Riordan. 2001. Efficacy of washing with a commercial flat-bed brush washer, using conventional and experimental washing agents, in reducing populations of *Escherichia coli* on artificially inoculated apples. J. Food Prot. 64:159–163.
2. Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barret, J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA 269:2217–2220.
3. Blanchard, H. 1999. *E. coli,* toxigenic, apple cider—USA (Oklahoma). [Internet, WWW], ADDRESS: http://www.healthnet.org/programs/promed.html.
4. Centers for Disease Control and Prevention. 1996. Outbreak of *E. coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice—October 1996. Morb. Mortal. Wkly. Rep. 45:975.
5. Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and Cryptosporidiosis associated with drinking unpasteurised apple cider—Connecticut and New York, October 1996. Morb. Mortal. Wkly. Rep. 46:4–8.
6. Dingman, D. W. 1999. Prevalence of *Escherichia coli* in apple cider manufactured in Connecticut. J. Food Prot. 62:567–573.
7. Duncan, D. W., and W. E. Razzell. 1972. *Klebsiella* biotypes among coliforms isolated from forest environments and farm produce. Appl. Microbiol. 24:933–938.
8. Fett, W. Personal communication.
9. Food and Drug Administration. 1996. Current science and technology on fresh juices; notice of meeting. Fed. Regist. 61:60290.
10. Food and Drug Administration. 1998. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice. Fed. Regist. 63:20450–20486.
11. Food and Drug Administration. 2001. FDA publishes final rule to increase safety of fruit and vegetable juices. [Internet, WWW], ADDRESS: http://www.fda.gov/bbs/topics/NEWS/2001/NEW00749.html.
12. Fratamico, P. M. Personal communication.
13. Hancock, D. D., T. E. Besser, D. H. Rice, E. D. Ebel, D. E. Herriott, and L. V. Carpenter. 1998. Multiple sources of *Escherichia coli* O157:H7 in feedlots and dairy in Northwestern USA. Prev. Vet. Med. 35:11–19.
14. Iwasa, M., S. Makino, H. Asakura, H. Kobori, and Y. Morimoto. 1999. Detection of *Escherichia coli* O157:H7 from *Musca domestica* (Diptera: Muscidae) at a cattle farm in Japan. J. Med. Entomol. 36:108–112.
15. Janisiewicz, W. J., W. S. Conway, M. W. Brown, G. M. Sapers, P. Fratamico, and R. L. Buchanan. 1999. Fate of *Escherichia coli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. Appl. Environ. Microbiol. 65:1–5.
16. Kobayashi, M., T. Sasaki, N. Saito, K. Tamura, K. Suzuki, H. Watanabe, and N. Agui. 1999. Houseflies: not simple mechanical vectors of enterohemorrhagic *Escherichia coli* O157:H7. Am. J. Trop. Med. Hyg. Oct. 61:625–629.
17. Luedtke, A. N., and D. A. Powell. 2000. A comparative analysis of Ontario cider producers information sources and production practices. Agri-Food Risk Management and Communications Technical Rep. 15. [Internet, WWW], ADDRESS: http://www.plant.uoguelph.ca/safefood/micro-haz/cider-survey-May00.htm.
18. McClellan, M. R., and D. F. Splittstoesser. 1996. Reducing the risk of *E. coli* in apple cider. Food Technol. 50:174.
19. Miller, L. G., and C. W. Kaspar. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. J. Food Prot. 57:460–464.
20. National Food Processors Association. 2000. NFPA white paper—significance of bacteria on fresh and minimally processed produce. [Internet, WWW], ADDRESS: http://207.153.197.248/science/wp%5Fecoli.html.

21. Parish, M. E. 1997. Public health and nonpasteurized fruit juices. Crit. Rev. Microbiol. 23:109–119.

22. Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. 308:681–685.

23. Riordan, D. C. R., G. M. Sapers, and B. A. Annous. 2000. The survival of *Escherichia coli* O157:H7 in the presence of *Penicillium expansum* and *Glomerella cingulata* in wounds on apple surfaces. J. Food Prot. 63:1637–1642.

24. Sapers, G. M., R. L. Miller, M. Jantschke, and A. M. Mattrazzo. 2000. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of apples containing *Escherichia coli*. J. Food Sci. 65:529–532.

25. SAS. 1989. SAS/STAT. User's guide version 6, 4th ed. Statistical Analysis Systems Institute, Cary, N.C.

26. Splittstoesser, D. F., D. T. Queale, J. L. Bowers, and M. Wilkinson. 1980. Coliform content of frozen blanched vegetables packed in the United States. J. Food Saf. 2:1–11.

27. Steele, B. T., N. Murphy, G. S. Arbus, and C. P. Rance. 1982. An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice. J. Pediatr. 101:963–965.

28. Sumner, S. S., and D. L. Peters. 1997. Microbiology of vegetables, p. 87–114. *In* D. S. Smith (ed.), Processing vegetables: science and technology. Technomic Publishing Co., Inc., Lancaster, Pa.

29. Uljas, H. E., and S. C. Ingham. 2000. Survey of apple growing, harvesting, and cider manufacturing practices in Wisconsin: implications for safety. J. Food Saf. 20:85–100.

30. Wallace, J. S., T. Cheasty, and K. Jones. 1997. Isolation of vero cytotoxin-producing *Escherichia coli* O157 from wild birds. J. Appl. Microbiol. 82:399–404.

31. Wright, J. R., S. S. Sumner, C. R. Hackney, M. D. Poerson, and B. W. Zoecklein. 2000. A survey of Virginia apple cider producers' practices. Dairy Food Environ. Sanit. 20:190–195.

32. Zhao, T., M. R. S. Clavero, M. P. Doyle, and L. R. Beuchat. 1997. Health relevance of the presence of fecal coliforms in iced tea and leaf tea. J. Food Prot. 60:215–218.

33. Zhao, T., M. P. Doyle, and R. E. Besser. 1993. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in apple cider with and without preservatives. Appl. Environ. Microbiol. 59:2526–2530.